Instituto Juan March de Estudios e Investigaciones

51 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Mechanisms of Expression and Function of MHC Class II Molecules

Organized by

B. Mach and A. Celada

L. Adorini
J. M. Boss
A. Celada
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INTRODUCTION

Antonio Celada

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The idea of organizing the Workshop on "Mechanisms of expression and function of MHC class II molecules" in Madrid was to cover two objectives: first, to make the point of the recent discoveries in the field of MHC class II molecules. The second objective was to meet other Spanish colleagues working in this field. The major credit for the organization of this meeting is to the co-organizer, Bernard Mach, who helped me in contacting a selected list of brilliant and active investigators in the field of class II molecules. With his prestige, Bernard supported this meeting which, due to the small size and the good facilities of the Juan March Foundation, made for an easy interaction between participants. Without the effort of Bernard this meeting would not have been possible. Finally, I should thank all the participants who spent some of their busy schedules coming to Madrid and making the success of the meeting possible.

I would like to mention that Spanish research in the last 20 years has made a dramatic improvement. This is due, certainly, to the political and economical changes that have occurred with the return of democracy to Spain. The increase in the budgets for research programmes resulted in a large number of publications appearing in scientific journals, produced by groups working in Spain. Another important aspect to science is communication, and we are very glad that some private foundations such as Juan March decided to support with enthusiasm and organize these biology meetings that help the interactions between Spain and the rest of the scientific community. One of the results of this effort is that many of the invited scientists to the workshop made their first visit to Spain, where they could learn through the presentations carried out during the meeting that, although Spanish science still needs to develop, putting our emphasis on the quality of the research, we are travelling in the good direction.

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The small number of participants allowed a good interaction during the presentations, the coffee breaks and lunches. Many cooperative projects will start from this meeting, helping all of us to do in a better way one of the things that we enjoy: research. Also, for young participants, it was a unique opportunity to meet one of the best groups of scientists working in the field of class II molecules. I am quite sure that soon some of these participants will apply for training as post-docs in some of the labs that made the presentations.

The immune system has two functions: one is to recognize what comes from outside and what comes from inside, and the other is to induce a reaction that eliminates what is foreign. MHC class II molecules play a key role in the immune system. These molecules are necessary to present to T lymphocytes the peptides that come from the processed antigens. It is very important to define the mechanisms that regulate the expression of MHC class II molecules. Once these molecules have been produced, it is also necessary to know the different steps from the endoplasmic reticulum until the molecules of class II are loaded with peptides and expressed at the cell surface. Therefore, the understanding of the regulation of the expression and functional activity of these molecules is one of the major steps to understand correctly the immune system.

The expression of class II genes is tissue specific and inducible in some cells by cytokines such as interferon gamma (IFNγ). For instance, in B lymphocytes the expression is constitutive, in macrophages it is inducible by IFNγ and in some other cells it is not expressed. This represents a good opportunity to study tissue specific and inducible transcription factors as a way to better understand the regulation of gene expression.

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In the study of molecules such as IFNγ that induce the expression of class II molecules, the next step is to define the interaction with the cells at their surface. IFNγ-receptors are proteins in which, after the interaction with the ligands, two tyrosin-kinases, JAK 1 and JAK 2 become activated.

As a result of the ligand-receptor interaction, a cascade of second signals is produced inside the cell. Some of these signals induce in minutes the expression of some genes (early genes), such as Stat 1, that are required for the subsequent transcription of other genes directly related with the expression of class II molecules.

Upstream of all MHC class II genes there exist three relatively conserved sequences that play an essential role in the transcription regulation of these genes. Some binding proteins are defective in patients with combined immunodeficiency, a disease in which there is no MHC class II expression.

MHC class II molecules assemble with the Invariant chain in the endoplasmatic reticulum and are then moved to endosomal/lysosomal compartments. There, Ii is proteolytically digested but fragments of Ii, designed CLIP, remain in the groove of class II molecules and are removed by HLA-DM, a non-classical MHC class II molecule, so that peptide loading with antigenic peptides can proceed.

At the end of the workshop, I asked myself if we have answers to all the questions that this exciting workshop provoked. Obviously that is not the case, and not only that, but I think that we have more questions that remain open to new ideas, experiments, etc. This suggests to me that this interesting topic is alive and we will need the help of many scientist to answer all the questions.

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A. Molecular and Genetic Basis for MHC class II regulation

Chairperson: Bernard Mach

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1.- Receptor activation, signal transduction and activation of early genes

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JAKS, STATS AND SIGNAL TRANSDUCTION IN RESPONSE TO THE INTERFERONS AND OTHER CYTOKINES

J Briscoe, D Guschin, NC Rogers, D Watling, M Müller[1], BA Witthuhn[2], C Beadling, D Cantrell, F Horn[3], P Heinrich[3], JN Ihle[2], GR Stark[4] & Ian M Kerr.
Imperial Cancer Research Fund, London WC2A 3PX, UK, [1]IFA, Tulln, Austria, [2]St. Jude Children's Research Hospital, Memphis, USA, [3]Biochimie, RWTH, Aachen, Germany, [4]Cleveland Clinic Foundation, Cleveland, USA.

Recent work on the interferons (IFNs) has established that they signal through a novel type of signal transduction pathway involving activation of transcription factors at the cell membrane. Activation of these factors known as STATs (Signal transducers and activators of transcription) is through the JAK (Janus kinase) family of protein tyrosine kinases. It is becoming increasingly clear that JAKs, STATs and similar pathways are utilised by a wide variety of cytokines and growth factors (reviewed Darnell *et al.* 1994, Ihle & Kerr 1995 and Schindler & Darnell 1995). Recent developments in our knowledge of the IFN response pathways will be briefly reviewed. The use of mutant cell lines defective in the JAKs and STATs in the analysis of these and other pathways will be described. Some contrasting results obtained with kinase-negative mutants of JAK1 and JAK2 in the dissection of the IFN and IL-6 responses will be presented.

The IFNs, first discovered as antiviral agents, can inhibit cell proliferation, promote differentiation and affect cell function particularly in relation to the immune response. There are three major antigenic types of human IFN α, β and γ. The αβ (TypeI) IFNs are thought to share the same cell membrane receptor(s) and induce a very similar set of polypeptides whereas IFN-γ (Type II) has a separate receptor and induces a distinct but overlapping set of polypetides. Induction is transient and is followed, to a variable degree in different cell types, by a refractory state. For both the IFN-α and the IFN-γ receptors two subunits have been cloned. Ligand binding triggers receptor dimerisation/oligomerisation. Tyrosine phosphorylation and activation of the JAKs, the receptors and STATs all occur in receptor complexes at the cell membrane. Activated STATs dimerise and, with or without additional factors, migrate to the nucleus to activate transcription through related response elements.

JAKs and STATs

The JAK family of non-receptor protein tyrosine kinases has four mammalian members JAK1, JAK2, JAK3 and Tyk2 and a Drosophila homologue 'hopscotch'. JAK1, JAK2 and Tyk2 appear universally expressed whereas the expression of JAK3 is restricted to cells of the immune system. Each is approximately 130kDa and has a C-terminal protein kinase domain, an adjacent kinase-like domain and five further domains with amino acid similarity within the family extending to the N-terminus. These additional domains likely mediate the protein/protein interactions governing the multiple roles of the JAKs in different signal transduction pathways. Seven STATs (1 to 6 including 5A and B) have been cloned. They range from about 80 to 110 kDa. Each has an SH2 and SH3 group. Activation involves phosphorylation of a conserved tyrosine just C-terminal to the SH2 domain. Homo- or heterodimerisation of the STATs, with or without additional polypeptides, yields a spectrum of transcription factors with differing affinities for a family of response elements.

Mutants in the interferon response pathways

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IFN-inducible promoters driving drug-selectable or cell-surface markers have been used to obtain and complement mutants in the IFN-αβ and -γ response pathways. Recessive mutants in eight complementation groups which affect one or other or both of the major IFN response pathways have been isolated. All have now been complemented. The complementing genes include JAK1, JAK2 and Tyk2, STATs 1 and 2 and ISGF3γ and subunits of the IFN-α and -γ receptors. In addition to identifying the role of the JAKs and the essential nature of each of these components in the IFN response pathways, the mutants are being used in the analysis of the responses to other cytokines and growth factors and provide a negative background for structure function analysis of the complementing gene products.

Kinase-negative JAKs

Kinase-negative mutants of JAK1 and JAK2 can act as dominant negative inhibitors of the IFN-αβ and IL-6 responses. In contrast, in the IFN-γ response, kinase-negative JAK1 can sustain substantial IFN-inducible gene expression. This indicates a structural as well as an enzymic role for JAK1 in the IFN-γ response. Despite their ability to sustain substantial IFN-γ-inducible gene expression the kinase negative JAK1 mutants do not sustain an IFN-γ-mediated antiviral response to encephalomyocarditis or Semliki Forest viruses. Accordingly although the activation of STAT1 is necessary for the antiviral response to IFN-γ it is not sufficient. It appears that a second JAK1 dependent signal/pathway is required (Briscoe *et al.* 1995) and, most importantly, that the STATS may not be the only targets of JAK activation.

Activation of JAKs and STATs in response other ligands

The JAKs and STATs are phosphorylated/activated in response to a wide variety of cytokines and growth factors. A major role for JAK1 and the non equivalence of the JAKs in the IL-6 response pathway has been clearly established using the mutant cell lines (Guschin *et al.* 1995). In the EGF system, STAT activation appears to occur independently of the JAKs (Leaman *et al.* 1995) This leaves us with the interesting question as to the role of the activated JAK1 in the EGF system. Moreover these and additional data suggest that STAT selection/activation may be governed in different ways in different response pathways depending on the nature of the individual receptor complexes involved.

Recent developments

Some recent developments of interest include: The modulation of STAT activity by serine phosphorylation emphasising the likely importance of cross talk between the JAK/STAT and other response pathways. The association of IRS1 and 2 with the JAKs and their activation by a number of cytokines and growth factors. The reported JAK/STAT activation in response to angiotensin, with the implication of the possible involvement of JAK/STAT pathways downstream of the seven transmembrane receptor family. The interaction of STATs with other transcription factors.

Overall, JAKs and STATs are activated by a wide variety of ligands. Hardly surprisingly, there is cross talk between the JAK/STAT and other pathways. The JAKs are not the only mediators of STAT activation and it may well be that the STATs are not the only targets of JAK activation. There is much yet to do.

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THE IFNγ RECEPTOR AND CYTOKINE SIGNALING IN MICE WITH TARGETED DEFICIENCIES IN JAK-STAT PATHWAY COMPONENTS.

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IFNγ is an important cytokine that exerts its pleiotropic effects on cells by interacting with a species specific receptor that is ubiquitously expressed on nearly all cells. Functionally active IFNγ receptors consist of two polypeptides: a 90 kDa α chain responsible for ligand binding, ligand trafficking and signal transduction and a 62 kDa β chain that is obligatorily required for IFNγ signaling. We have completed a detailed structure function analysis of the intracellular domains of the two IFNγ receptor subunits and now have a clear picture of the IFNγ signal transduction process. In unstimulated cells, the IFNγ receptor α and β subunits are not preassociated with one another but constitutively associate, through their intracellular domains, with inactive forms of distinct Janus family tyrosine kinases. A membrane proximal four amino acid sequence in the intracellular domain of the receptor α chain forms the site of attachment for JAK-1 and a twelve amino acid sequence in the membrane proximal half of the receptor β chain intracellular domain forms the attachment site for JAK-2. Upon binding to homodimeric IFNγ ligand, two IFNγ receptor α chains dimerize forming a complex to which the receptor β chain subunit subsequently binds. In this ligand assembled complex the intracellular domains of the receptor subunits are brought into close proximity with one another together with the kinases that they carry. JAK-1 and JAK-2 transactivate one another and then phosphorylate a specific tyrosine containing sequence (YDKPH) in the membrane distal portions of paired receptor α chain intracellular domains thereby forming a paired set of specific docking sites on the activated receptor complex for the latent cytosolic transcription factor, Stat1. Receptor bound Stat1 proteins are then activated by tyrosine phosphorylation and form a stable Stat1 homodimer that translocates to the nucleus where it initiates transcription of IFNγ inducible genes. To explore the physiologic significance and specificity of the Stat1 dependent IFNγ signaling pathway, we have generated, by homologous recombination techniques, two strains of mice with genetic deficiencies of either Stat1 or JAK-1. The responsiveness of these mice to IFNγ and IFNα/β as well as to other cytokines that have been reported to activate Stat1 and/or JAK-1 will be discussed.

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2.- Use of genetics in the discovery of MHC class II transactivators

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THE MHC CLASS II TRANSACTIVATOR CIITA, A "MASTER REGULATOR" OF MHC CLASS II GENE EXPRESSION

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Precise regulation of MHC class II gene expression plays a crucial role in the control of the immune response. Both the constitutive and inducible modes of MHC class II expression are controlled essentially at the transcriptional level via the proximal MHC class II promoters [1]. Elucidation of the genetic defects of two forms of hereditary MHC class II deficiency by genetic complementation approaches lead to the identification of the two essential MHC class II regulators CIITA and RFX5 [2,3]. CIITA, a large protein of 1130 amino acids, does not seem to bind directly to the MHC class II promoters. Its N-terminal acidic domain, and to a certain extend the Pro/Ser/Thr rich domain, can be replaced by a viral acidic activation domain and still activate endogenous MHC class II transcription as shown by us and other laboratories. This may indicate that CIITA acts in a non-DNA binding co-activator like fashion.

In addition to its essential role in constitutive MHC class II expression, CIITA also plays a key role in the inducible MHC class II expression [4]. CIITA is itself differentially expressed and the level of CIITA expression seems to be directly correlated to MHC class II promoter activity (see also abstract by L. Otten et al). We could demonstrate that CIITA expression is both necessary and sufficient to activate MHC class II transcription in IFN-γ inducible cell lines and that IFN-γ induced MHC class II expression is mediated via the induction of CIITA expression. These observations identify CIITA as a "master control factor" of MHC class II expression and indicate that to an important degree MHC class II expression is actually controlled at the level of CIITA expression [4]. This obviously re-focuses the interest in MHC class II regulation towards the regulation of CIITA itself. Unexpectedly, the CIITA gene is controlled by at least four independent promoters. It is of particular interest that one promoter primarily

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controls constitutive expression in B cells, while another is largely responsible for IFN-γ inducible expression.

An interesting model system for the regulation of CIITA induction by IFN-γ may be presented by the murine RAG cell line. RAG displays a defect in the induction of MHC class II by IFN-γ, which can be complemented by human chromosome 16 (Bono et al, PNAS 88: 6077-6081, 1991). Human CIITA, which is itself located on chromosome 16, is able to induce MHC class II expression in RAG when expressed constitutively. Furthermore, RAG is defective in the induction of CIITA mRNA. The study of the CIITA-induction defect in RAG should therefore provide interesting insight into the mechanism(s) controlling CIITA induction by IFN-γ.

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Structure and function of RFX, the X box binding factor mutated in MHC class II deficiency

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The major control region governing cell specific and inducible expression of MHC class II genes is a 150 bp promoter proximal region situated immediately upstream of the transcription initiation site. The promoter proximal regions of all MHC class II genes, from all species examined. share four highly conserved cis-acting sequences referred to as the S, X, X2 and Y boxes. The identification and cloning of the trans-acting regulatory factors controlling the activity of these cis-acting elements has been greatly facilitated by a genetic approach, namely the analysis of cells exhibiting a regulatory defect in MHC class II gene expression (for reviews see 1, 2). The majority of such cells are derived from patients suffering from a severe primary immunodeficiency called MHC class II deficiency, also referred to as the bare lymphocyte syndrome (BLS) (1, 2). Together with a number of experimentally generated regulatory mutants, cells from BLS patients have been classified in up to five complementation groups (A to E) believed to correspond to defects in five different trans-acting regulatory factors (1,2 and 3).

The gene mutated in group A encodes the MHC class II transactivator CIITA (see abstract by V. Steimle et al.) (4, 5). Of the remaining complementation groups, three (B to D) are due to a deficiency in the binding activity of the X box binding complex RFX (1, 2, 6). RFX is a heterodimeric protein consisting of two subunits, p36 and p75 (6).

The gene (RFX5) encoding the p75 subunit was cloned by a genetic complementation approach (7). RFX5; is mutated in all BLS patients in complementation group C (1, 2, 7). RFX5 derives its name from the fact that it was the fifth member of a growing family of DNA binding poteins containing the novel RFX DNA binding motif (8). Additional RFX family members have now been identified in S. cerevisiae, S. pombe, C. elegans and D. melanogaster, indicating that the RFX motif has been conserved throughout the eukaryotic kingdom, and has been recruited into proteins having a surprisingly diverse range of biological functions (8).

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The p36 subunit of the RFX complex has been purified by affinity chromatography, and full length cDNAs encoding this subunit have now been isolated on the basis of peptide sequences derived from the purified protein. p36 is a novel protein exhibiting no homology to any other known proteins. Surprisingly, it does not contain the characteristic RFX DNA binding motif.Whether mutations in the p36 gene account for one of the other RFX deficient complementation groups (B or D) is presently under investigation.

RFX plays a central role in the occupation of MHC class II promoters in vivo because it binds cooperatively with two other MHC class II promoter binding factors, the X2 box binding complex X2BP and the Y box binding factor NF-Y (1, 2). These cooperative binding interactions greatly enhance the afinity of RFX, X2BP and NF-Y for their respective target sequences, thereby permitting their recruitment even to promoters having only very low affinity binding sites. In addition, we have now also shown that these cooperative binding interactions are highly specific. For example, RFX can not be replaced by other X box binding proteins such as RFX1, and X2BP can not be replaced by other X2 box binding proteins such as c-jun/c-fos. Consequently, these specific protein-protein interactions ensure that RFX, X2BP and NF-Y, rather than other proteins capable of recognizing the X, X2 and Y boxes, are recruited selectively to MHC class II promoters. Finally, a comparisson between extracts from cells that express CIITA (Raji, HeLa induced with interferon-γ, HeLa transfected with CIITA) and cells that lack CIITA (RJ2.2.5, uninduced HeLa) has demonstrated that the cooperative binding interactions between RFX, X2BP and NF-Y are independent of the expression of CIITA. CIITA does therefore not appear to stabilize binding of these proteins to the promoter.

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Mutant B cell lines expressing HLA-DR and -DQ but not -DP: evidences for specific transcriptional regulation of HLA-DPA and DPB genes.

M. Coiras, A.M. Álvarez, C. Nombela, M Sánchez and J. Arroyo.

A series of mutant B lymphoblastoid cell lines had been previously obtained in our laboratory by means of ICR191 treatment and selection with HLA-DPw2 specific CTLs from the HLA haploid cell line 45.1. None of these mutants expresses HLA-DPw2 in spite of the fact that normal levels of HLA-DR and DQ are expressed. Northern blot analysis indicated a marked decrease in the levels of DPA or/and DPB mRNAs in these mutants.

Transient heterokarions between the LCL 721.180, that lacks the structural genes for HLA class II antigens, and different HLA-DP mutants were obtained. HLA-DP expression was recovered in some of the fusions, indicating that lack of HLA-DP expression in these mutants results from a defect in (or operating through) an HLA DPA or DPB specific trans-acting factor. Cloning and sequencing of the upstream DPA (from -1 to -258) and DPB (from -1 to -264) regions, in mutants unable to recover HLA-DP expression after fusion with 721.180, did not reveal differences with the parental cell line (45.1)

To test wether the defect in HLA-DP expression could be restored by different cytokines, mutant LCLs were treated with IFN-γ, IL-4, IL-1, IL6, TNFα and TNFβ. None of these cytokines, alone or together, were able to induce expression of HLA-DP in the mutants, which suggests that the defective factors are not only required for constituve expression but also for inducible expression of these genes.

We have examined proteins binding to the consensus sequences of the DPA and DPB promoters (X, Y, J and S boxes), in those mutants which recovered expression of HLA-DP after fusion with the LCL 180. Gel retardation assays, using oligonucleotides specific for X,Y,J and S boxes from HLA-DPA and DPB genes and nuclear extracts from mutants and parental cell line were carried out, but no differences were detected with any of the probes.

Our data clearly suggest the existence of trans-acting factors regulating specifically transcription of HLA-DPA and DPB genes, altough more studies will be necesarily to map precisely the defect responsible for its phenotype.

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3.- Mechanism of MHC class II promoter activation

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CONTROL OF KNOWN GENES INVOLVED IN CLASS II MHC ANTIGEN PRESENTATION: MOLECULAR ANALYSIS AND BIOLOGIC IMPLICATIONS.

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Expression of MHC Class II genes, the associated invariant chain (Ii) gene and the DMA/DMB genes is studied in this report and the biologic implications and consequences of their dysregulation are analyzed in mice bearing null alleles.

The class II transactivator (CIITA) and the RFX DNA-binding protein (both identified by Dr. Bernard Mach's group) can respectively restore class II MHC expression on cells derived from two distinct classes of Bare Lymphocyte Syndrome (BLS). It has been demonstrated by several groups that defects in both CIITA and RFX can lead to a lack of constitutive as well as IFN-γ induced class II MHC expression. Furthermore, introduction of CIITA can restore class II MHC gene expression in IFN-γ mutants that are selectively defective in class II MHC expression.

Our group (in collaboration with Drs. George Stark and Jeremy Boss) and Dr. Flavell's independently have found that CIITA not only regulates the class II MHC genes, but also coordinately regulates the expression of all known genes necessary for class II MHC mediated antigen presentation, including the Invariant chain gene, and the DMA/DMB genes. Similar findings have now been found for the RFX protein by analyzing an IFN-γ mutant cell line that is defective in RFX binding activity.

CIITA is an extremely potent activator of all these promoters, and its transactivates through the immediate proximal promoter of class II MHC genes. However, its mode of action is unknown. Three studies are presented here to address this point:

1. A structure-function analysis was undertaken to understand the possible mode of CIITA action. The N-terminal acidic domain alone is not sufficient to activate MHC - class II expression but requires additional proline/serine or threonine rich domains. Functionally essential guanine nucleotide binding motifs are identified, including a phosphate binding motif, and guanine binding motif. Another indispensable domain lies within the C-terminal region downstream of the BLS-2 deletion. Mutation of any of these domains abolished most of CIITA's transactivation. The necessity for guanine nucleotide binding motifs is especially interesting, and suggests that CIITA may constitute a new class of transactivator bearing such domains.

2. In vivo footprinting shows the HLA-DRA, Ii and DMB promoters are unoccupied by mutant cells lacking CIITA. Introduction of CIITA into the defective cell line reconstitutes all promoter loading to normal levels. However, it has been previously shown by Dr. Laurie Glimcher that the B cell line RJ2.2.5 in which CIITA is also mutated has occupied DRA promoter. This suggests that the mutations in these two cells are distinct and that CIITA may function at two separable stages: a) promoter occupancy and b) transcription activation.

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3. CIITA transactivation of HLA-DRA requires intact W, X and Y promoter elements which confirms data independently generated by Drs. Laurie Glimcher and Jeremy Boss. This report shows that additionally, CIITA transactivation requires that these elements be specifically aligned. In vitro DNA binding studies demonstrate that the assembly of X1, X2, and Y box proteins is indistinguishable in the presence or absence of CIITA, suggesting that CIITA does not directly affect the binding of proteins to DNA. Antibodies specific for CIITA do not show any reactivity against the in vitro DNA/protein complexes. This indicates that CIITA may not be tethered directly to the X and Y DNA/protein complexes.

In an additional study to identify biologic regulators of genes involved in class II MHC-mediated antigen processing, it was found that nitric oxide is a potent down-regulator of DR and Ii promoters. Cells prepared from mice with a defect in nitric oxide production exhibit significantly activated class II and Ii promoters. The mechanism of this down-regulation and the biologic implications of this will be discussed.

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Activation of Major Histocompatibility Complex Class II Genes

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Class II major histocompatibility complex (MHC) genes are regulated for the most part in a coordinate manner. In B cells, expression of class II genes begins early during lineage commitment and peaks at the mature B cell stage. Expression in B cells is controlled by a compact conserved upstream regulatory region consisting of 4 discrete elements, the W/Z/S, X1, X2, and Y boxes. Of these elements, the X box appears to be the central element and is required for all aspects of class II expression. The use of cell lines derived from patients exhibiting the bare lymphocyte syndrome, a disease in which transcription of the class II MHC genes does not occur, identified two factors that were required for class II expression: RFX and CIITA. While RFX is clearly a class II specific DNA-binding protein that binds to the X1 box (1,2), CIITA does not appear to interact directly with DNA (3,4). We recently identified the X2 box factor as X2BP. Purification of X2BP showed that it was a heterodimer composed of proteins of 46 and 120 kDa in mass. Because the X2 site is homologous to cAMP and TPA responsive elements, a series of Western blots were carried out on X2BP using antiseras directed against members of this family of proteins. These experiments suggested that X2BP was related to the CREB family of proteins due to the observed cross reactivity to anti-rat CREB antisera. The correlation of X2BP binding activity and its role in class II expression was demonstrated from experiments showing stable RFX-X2BP binding to X box DNA. Using chimeric activators co-transfected into B cells with reporter genes containing binding sites for these activators, we proposed a mechanism for class II expression and a function for CIITA (3,5). In this model, CIITA interacts with the DNA bound factors and activates expression through a potent acidic activation domain. To investigate CIITA with regard to its interactions with class II specific factors and the transcriptional machinery, a series of internal deletions were created. This mutagenesis showed that the majority of CIITA is critical for function. Deletion of sequences within the N-terminal ser/pro/thr-rich region surprisingly had little effect on the ability of CIITA to trans-activate a class II dependent reporter construction; while, in-frame deletions within the C-terminal region abolished function. A gene encoding a subunit of RFX, RFX5, was recently identified (6); however, the mechanism by which this protein functions is unknown. To determine if RFX5 contains an activation domain, GAL4 DNA-binding domain-RFX5 chimeric constructions were created and tested for activity. These experiments showed that while the entire RFX5 protein shows no transactivation potential, specific domains within the protein can activate transcription. The ability of the GAL4-RFX5 constructions to interact with class II bound factors was also tested and suggested that an internal region of RFX5 could interact with other class II specific factors. Together, these studies support our model of a compact regulatory region that requires multiple protein-protein interactions in order to activate transcription of MHC class II genes.

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Identification and regulation of IAX, a tissue specific and IFNγ inducible transcription factor that regulates the expression of MHC class II gene I-Aβ

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We have identified the cis-acting elements within the promoter of the MHC class II gene I-Aβ that are necessary for the expression of this gene in macrophages as well as B cells. These important *cis-acting* elements lie within 124 bp upstream of the transcription start site. There are three elements named the W, X and Y boxes that are important for the expression of the I-Aβ gene. These cis-acting elements resemble others found in the promoters of other MHC class II genes.

The X box is an important element for the expression of IAβ MHC class II gene and appears to be the binding site for two set of proteins. We have identified a novel protein that binds to the upstream side of the I-Aβ X box (X_1) and cloned the gene for this protein. The gene has been named IAX. There is a 72% identity within the human and murine gene. The sequence of IAX shows no homology to previously isolated genes and the putative protein sequence does not resemble any of the known DNA binding proteins. The binding site of IAX show an important homology with the structure of helix-turn-helix. Antisense constructs of the IAX gene were shown to lower the level of expression from the I-Aβ promoter.

Using a polyclonal antibody against the recombinant IAX, two bands of about 36 kD were detected by Western blot analysis. In mouse, mRNA and protein are present in B cells and macrophages, but are undetectable in T cells and fibroblats. Furthermore, Interferon γ induces IAX mRNA and protein expression in macrophages and fibroblasts. Thus, the pattern of expression and regulation of IAX gene is similar to modulation of MHC class II gene expression. However, expression of IAX precedes IAb expression. The induction of I-Aβ mRNA by IFNγ on macrophages starts at

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8 hours and during the first 30 minutes is dependent of protein synthesis. By contrast, induction of IAX mRNA by IFNγ is very fast (maximum at 1 hour) and is independent of protein synthesis.

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THE MHC CLASS II EA PROMOTER REQUIRES TFIID BINDING TO INITIATOR SEQUENCES.

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The MHC Class II Ea promoter is dependent on the presence of conserved upstream X-Y boxes and of Initiator sequences. In vitro transcription analysis of the Inr region with linker-scanning mutants pinpoint a functionally essential element that shows homology to the TdT Inr; the key sequence, between pos. +5 and +12, is located within a transcribed area. Inr-binding proteins LBP-1/CP2 and TIP -a TdT Inr-binding protein unrelated to YY1-recognize the Ea Inr, but their binding does not coincide with Ea Initiator activity. A good correlation is found with binding of immunopurified holo-TFIID to this element. TFIID interacts both with Ea TATA-like and Inr sequences, but only the latter is functionally relevant. Unlike TBP, TFIID binds in the absence of TFIIA, indicating a stabilizing role for TAFs in Ea promoter recognition. Comparison with other MHC Class II promoters suggests common mechanisms of start site(s) selection for this gene family. Protein-protein interaction assays between TBP (and TFIID) and the trimeric CCAAT binding protein NF-Y identify short peptide sequences in the evolutionary conserved domains of both NF-YB and NF-YC that are able to contact TBP directly. They show resemblance between them and with other TBP-interacting protein surfaces. These data support the hypothesis that TFIID bypasses TATA box binding by making protein-protein interactions with NF-Y and direct contacts with DNA sequences at the Inr.

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4.- Functional role of MHC class II transactivators

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IMMUNOMODULATION VIA MHC CLASS II TRANSACTIVATORS

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The immune response is under the control of the precise pattern of regulation of MHC class II genes. The tight control of this regulation, in professional and nonprofessional APCs, determines their immunogenicity and hence T cell activation, in both normal and pathological situations. The possibility of novel forms of experimental immunomodulation through certain key regulators of MHC class II expression gave a strong impetus to the elucidation of the molecular mechanisms involved. From the study of primary immunodeficiency syndromes, our laboratory was able to identify certain key regulatory genes, responsible for the control of MHC class II gene expression (1-3). Both RFX5 and CIITA are MHC class II transactivators that exhibit two unusual and interesting properties : they are absolutely essential for MHC class II expression and their effect is essentially restricted to MHC class II genes (1-3). On that basis, these two novel transactivators, as well as agents capable of modulating their activity, could be used for both up and down regulation of MHC class II expression and thus for immunomodulation.

Following transfection, expression of CIITA in a variety of MHC class II negative cell types leads to expression of MHC class II, as well as Ii, DMA and DMB molecules. Interestingly, however, these nonprofessional APC's exhibit a novel phenotype (4) : while capable of efficiently presenting synthetic peptides to CD4 T lymphocytes, they are not capable of presenting exogenous protein antigens. The processing defect responsible for this behavior of CIITA transfectants can be corrected by interferon gamma. This points to yet an additional level of complexity of the complex mechanism of MHC class II dependant antigen presentation (4). Specific endosomal proteases, inducible by interferon gamma but CIITA independant, are possible candidates for the observed processing defect. In contrast, we suspect on the basis of other experiments, that expression of MHC class II molecules by such nonprofessional APCs is sufficient for presentation of endogenous protein antigens to CD4 T cells. These transfection experiments with the transactivator CIITA have also shown that these cells can behave as highly efficient "presenter" of exogenous synthetic peptides (5), with potential implications in vaccination protocols.

Experimental down regulation of MHC class II expression is an interesting objective in certain pathological situations, primarily for the purpose of reducing T cell activation. Both RFX5 and CIITA genes and proteins are candidates to achieve such down regulation. We have constructed a number of mutant forms of these two regulatory genes for the purpose of generating dominant negative variants. Several dominant negative mutants of CIITA have been obtained. They are capable of reducing expression of all endogenous MHC class II genes in B cell lines (6). Such mutants represents obvious tools for in vivo reduction of MHC class II expression in selective tissues.

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Interleukin-10 inhibits the transport of mature MHC class II glycoproteins from the MIIC compartment to the plasma membrane in human monocytes.

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Interleukin-10 inhibits antigen specific proliferative T cell responses when human monocytes are used as antigen-presenting cells. Although both T cells and monocytes express IL-10 receptors, the inhibition of T cell proliferation under these conditions is mediated through effects of IL-10 on the monocyte. IL-10 inhibits the secretion of proinflammatory cytokines and hemopoietic growth factors by activated monocytes. In addition, it downregulates the constitutive or IFN-γ induced expression of the costimulatory molecules CD80 and CD86, the cell adhesion molecule ICAM-1 and the antigenic peptide binding MHC class II molecules.

In the present study we investigated the mechanism by which IL-10 mediates its inhibitory effect on MHC class II expression by human monocytes. IL-10 affects a posttransciptional event in class II synthesis as equivalent levels of steady state MHC class II DRA, DRB and invariant chain mRNA were present in mock treated and IL-10 treated monocytes. Biochemical analysis of MHC class II proteins in IL-10 treated monocytes suggest that levels of polypeptide chain synthesis and subunit assembly are not affected. In addition, pulse chase analysis has indicated that the dissociation kinetics of the MHC class II associated invariant chain are similar in IL-10 and mock treated monocytes. Furthermore, equivalent levels of SDS stable heterodimers could be demonstrated in control and IL-10 treated cells. However, biosynthetically labelled MHC class II heterodimers were not affected by neuraminidase treatment of IL-10 treated intact monocytes, indicating that these molecules did not appear at the cell surface. In addition, we were unable to detect newly synthesized MHC class II molecules at the cell surface of IL-10 treated cells by prebinding MHC class II specific mAbs before cell lysis. Finally, intracellular staining confirmed that MHC class II molecules were retained in a compartment resembling the MIIC. These results indicate that trafficking of mature MHC class II molecules from MIIC to the plasma membrane is regulated, and that it can be inhibited by IL-10.

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Mechanistic Study of Inhibition of Interferon-γ-induced MHC Class II Gene Expression By Interferon-β

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IFN-β can inhibit the induction of MHC class II expression by IFN-γ in many cell types. This is a well-documented observation and is of clinical significance in many human diseases including multiple sclerosis, since IFN-β has been approved by FDA to be the first drug to treat multiple sclerosis patients and the treatment mechanism is suspected to be related to the ability of IFN-β to inhibit IFN-γ-induced MHC class II expression. However, the mechanism of this inhibition is unclear. We study the role of individual ISRE-ISGF3 signaling components in the pathway by which IFN-β inhibits MHC class II expression. Electrophoretical mobility shift assay result indicates that Stat 1α(p91) remains fully available for IFN-γ signaling, after treatment with saturating concentration of IFN-β, thus excluding the possibility that IFN-β might function to sequester common signaling component for IFN-γ signaling. We found that IFN-β inhibition of IFN-γ-induced MHC class II expression is totally abolished in mutant cell line missing functional ISGF3γ(p48) while the inhibition by TGF-β of IFN-γ-induced MHC class II expression is intact. These results strongly suggest that an IFN-β-induced product, expressed via the ISGF-ISGF3-dependent pathway, is responsible for the inhibition of IFN-γ-induced MHC class II expression.

Recently, a novel factor termed CIITA has been identified as essential for IFN-γ-induced MHC class II transcription. We studied the status of IFN-γ-induced CIITA mRNA accumulation and CIITA-driven transactivation in IFN-β-treated cells in this inhibition. IFN-β-treatment did not suppress IFN-γ-induced accumulation of CIITA mRNA. After cells were stably-transfected with CIITA, endogenous MHC class II genes were constitutively expressed, and MHC class II promoters, delivered by transfection, were actively transcribed in CIITA-expressing cells. Expression of these promoters was significantly impaired by pretreatment with IFN-β. Taken together, results of these experiments suggest that inhibition of MHC class II gene expression in IFN-β-treated cells requires expression of gene(s) directed by the ISGF3-Interferon-Stimulated Response Element (ISRE) pathway, and that these gene product(s) act downstream of CIITA mRNA accumulation to block CIITA-driven MHC II gene expression.

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B. Mechanism of antigen presentation by MHC class II molecules

Chairperson: John Trowsdale

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1.- Role of Ii and DM in MHC class II trafficking and in peptide loading

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Organisation and Function of Class II Genes and Molecules.

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The class II region of the human MHC contains all of the known class II genes: *DM, DN/DO, DP, DQ, DR* as well as the antigen processing components *TAP/LMP* and only one gene not obviously associated with the immune system, *RING3*. As an approach to understanding linkage disequilibrium and recombination in relation to polymorphism of the region we are sequencing the class II region in collaboration with Stephan Beck[1]. To date, the sequence of the *DP-DQ* region has almost been completed.

Functions of the genes are being investigated. *HLA-DM* has provoked considerable interest since an intact DM molecule is necessary for exchanging peptides in the DR peptide-binding groove. Expression of the DM genes is inducible with γ–interferon and the DM products are found in tissues expressing other class II structures. The HLA-DM α and β sequences are only weakly related to those of DR, indicating that DM genes were derived from class I/II primordial genes very early, but the peptide sequence may fold into a structure similar to class II[2]. It is not obvious from computer modelling whether DM molecules contain a peptide-binding groove.

In collaboration with Hans Gueze's laboratory, we have shown that DM molecules are not expressed on the cell surface at steady state but are localised in so-called MIIC vesicles, where peptides are thought to be loaded onto class II molecules[3]. We have studied whether DM molecules interact with DR. After *in vitro* translation we could demonstrate association of DR molecules with the invariant chain but not with DM(unpublished data). By in vivo work, in contrast, using low pH and detergents such as Digitonin we demonstrated co-precipitation of the two molecules[4]. This association was not observed when the Mellins DRA mutant $DR3^{ser96}$ was used, which results in an extra N-linked glycosylation site. This mutant has an *in vivo* phenotype like that of DM mutants and is consistent with the idea that the bulky carbohydrate group interferes with a DM/DR interaction. These findings have been complemented by in vitro data on peptide exchange using DM protein made in Baculovirus.

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HLA-DM and MHC Class II-Restricted Antigen Processing

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MHC class II molecules are normally occupied by a large range of peptides derived from proteins degraded in the endosomal-lysosomal system. In a certain set of human mutant cell lines, they are instead occupied by a single nested set of peptides derived from the invariant chain called Class II-associated Invariant chain Peptides (CLIP). Complexes of class II molecules with CLIP constitute an intermediate in class II processing in wild-type cells which accumulate in the mutant cell lines because they lack one or both subunits of the HLA-DM molecule. Restoring HLA-DM expression in the mutant cell lines repairs the defect and normal class II peptide loading is restored. Purified HLA-DM molecules can induce CLIP dissociation from class II αβCLIP complexes and facilitate the binding of antigenic peptides in solution. In wild-type cells, HLA-DM is localized intracellularly in the lysosome-like MHC-class II containing compartments (MIICs). Current models suggest that class II αβ-invariant chain complexes enter the endosomal-lysosomal pathway, invariant chain degradation generates αβCLIP complexes, and HLA-DM catalyzes CLIP dissociation and peptide loading in the MIICs.

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INTRACELLULAR FORMATION AND EXTRACELLULAR ACTIVATING FUNCTION OF MHC CLASS II-PEPTIDE LIGANDS FOR αβ TCR

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Major histocompatibility complex (MHC) class II molecules must be assembled from their α and β subunits and transported to endocytic organelles to serve their function in the capture and cell surface display of peptides derived from exogenous protein antigens. The assembly of the class II binding site in the endoplasmic reticulum and function of the binding site in endosomes creates several problems that are solved through the interaction of newly synthesized class II molecules with a nonpolymorphic, non-MHC encoded type II glycoprotein called the invariant chain (Ii). This molecule assembles with newly synthesized αβ heterodimers in the ER. Structure-function analysis using truncation mutants has revealed that the region encoded by exon 3 of Ii is critical to this interaction [1-3]. This exon encodes the CLIP peptide found associated with class II molecules produced by cells deficient in DM expression [4, 5]. CLIP is central to the contribution of Ii to the physiological secretory pathway behavior of class II, including proper dimer formation in the ER, avoidance of aggregation in this organelle, transport through the Golgi complex, and inhibition of inappropriate binding to protein ligands encountered in the secretory pathway [3]. CLIP mediates these diverse effects by interacting directly with the class II binding site, providing some of the structural stabilization previously reported to be characteristic of peptide binding to empty class II molecules [6, 7] and sterically inhibiting association of other ligands with these stabilized sites. Another region in the Ii lumenal domain mediates trimerization, which appears crucial for effective targeting to early endosomes via cytoplasmic sorting signals. Ii-class II complexes next traffic to late endosomes and then a lysosome-like compartment where the bulk of Ii is proteolyzed [8], and with the aid of DM, CLIP removed [9-11], permitting interaction with partially denatured proteins. These longer fragments are then trimmed to produce the typical class II-peptide complexes exported to the cell surface. These data support a modular structure-function relationship for Ii and reveal its contribution to multiple aspects of class II function.

A second presentation pathway independent of Ii relies on signals in the tail of the class II molecule to access endocytically processed antigens [12]. Recent work in our laboratory suggests that one important component of this signal lies in the tail of the class II β chain, and is highly homologous to one of the sorting signals previously identified in the tail of Ii [13-15]. This signal appears to control internalization of mature class II from the cell surface, creating a pool of class II primarily responsible for presentation of determinants that would be degraded too rapidly for effective capture in the MIIC, thus helping the immune system to extract maximum antigenic information from proteins.

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Once these peptide-MHC molecule complexes reach the surface, they are available for binding to the clonotypic αβ receptors on T cells. Recent studies have shown that small variations in the structure of the peptide or MHC component can create TCR ligands with partial agonist or antagonist properties. Our studies have shown that there is an altered pattern of early TCR-induced tyrosine phosphorylation in cells exposed to such variant ligands. This pattern involves as altered ratio of phosphorylated ζ chain isoforms, diminished CD3 chain phosphorylation, and recruitment of ZAP-70 to the TCR complex without accumulation of activated, phosphorylate ZAP-70 kinase [16]. Recent experiments examining anergy induction suggest that some downstream signals are propagated upon T cell exposure to variant ligands, and in studies using CD8+ CTL, putative "pure" TCR antagonists consisting of class I MHC molecules and a variant peptide have been found to cause ζ phosphorylation without other apparent phosphorylation events or induction of effector activities. These data suggest a model for ligand-induced TCR activation involving both kinetic and architectural constraints [17, 18].

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Mechanisms regulating peptide loading onto MHC class II molecules

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MHC class II molecules assemble with the Invariant chain in the endoplasmatic reticulum and are then storted to endosomal/lysosomal compartments. There, Ii is proteolytically digested but fragments of Ii, designated CLIP remain in the groove of class II molecules and need to be removed so that peptide loading with antigenic peptides can proceed. This is achieved by two distinct mechanisms.

1. Our studies show that the N-terminus of CLIP (81-104) interacts with an effective site close to the groove of class II molecules, resulting in self-release of CLIP. Interestingly, the isolated N-terminal fragment of CLIP (81-89) can perform this function and mediate not only the release of the CLIP (90-104) core fragment from the groove but also of a set of sub-optimally bound self-peptides, suggesting a peptide editing function.

2. However, for many class II molecules the self-release of CLIP is not efficient enough to allow loading of classs II molecules during their relatively short transit time through the endosomal/lysosomal compartments. HLA-DM is a non-classical MHC class II molecule that is known to facilitate peptide loading by accellerating CLIP release. Using in vitro approaches, we have found that sub-stoichiometric amounts of DM are also able to accellerate the loading process itself, following Michaelis-Menten kinetics, with Km values of about 1μM (reflecting the affinity of intermediary DR-DM complexes) and turnover rates of 5-20 molecules per minute depending on the class II allele and nature of the peptide. Similar to the effect of the CLIP N-terminus, DM also accellerates the release of a large set of sub-otimally bound self-peptides. In vivo this probably means that high affinity peptides, which may be less abundant in the respective loading compartment, get another chance to bind to class II molecules.

Thus, DM serves as an efficient catalyst for peptide loading with enzyme-like kinetics, and also performs peptide editing functions.

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Degradation of the MHC Class II-Associated Invariant Chain by Proteases of the Endocytic Route.

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MHC class II molecules bind and present peptides derived from antigens degraded in the endocytic pathway. Class II molecules are synthesized in the endoplasmic reticulum as heterodimers associated with the Invariant chain (Ii). Ii occupies the peptide-binding site of class II molecules through its CLIP region, precluding binding of antigenic peptides. Ii also contains signals in its cytoplasmic tail which direct the complex into the endocytic route. Once there, Ii is degraded by the action of one or more proteases, whose identification has remained elusive. Several *in vitro* and *in vivo* studies employing purified enzymes and specific protease inhibitors have implicated the Cathepsins B and D as major players in this process. Following degradation, the resulting fragments of Ii that contain the CLIP region remain associated with the class II dimers, and their release is promoted by the catalytic action of HLA-DM (or its mouse counterpart, H-2M). Eventually, antigenic peptides can bind to the class II dimers and are displayed at the cell surface. This process is regulated and involves several steps confined to specific compartments of the endocytic route. For instance, HLA-DM can probably displace fragments of Ii of discrete size, and such fragments will only occur after the class II-Ii complexes have traversed a compartment containing an appropriate protease (s). Similarly, substitution of CLIP by antigenic peptides takes place in the MHC class II compartment (MIIC), where acquisition of SDS stability by class II dimers and accumulation of HLA-DM have been reported.

We describe here that neither Cathepsin B nor D, but Cathepsin S is the major protease involved in the generation of the minimum CLIP-containing fragments that remain associated to HLA class II $\alpha\beta$ dimers after degradation of Ii. Treatment *in vitro* of $\alpha\beta$Ii complexes with pure preparations of this enzyme resulted in generation of short CLIP-containing peptides. A pure preparation of Cathepsin B could not degrade Ii. On the other hand, mice deficient in Cathepsin D (knock-out) showed normal maturation of MHC class II molecules, indicating that this enzyme is not a major player for degradation of Ii nor in generation of the bulk of antigenic peptides.

In studies carried out on mouse spleen cells, we observed that the degradation of Ii proceeds in a staged fashion, generating discrete fragments of Ii that remain bound to MHC class II heterodimers. In H-2^b mice, these complexes were stable in SDS at room temperature, and showed distinct mobility on SDS-PAGE. Treatment with Leupeptin or Concanamycin B resulted in accumulation of different such intermediates. The intermediates that accumulated in the presence of Leupeptin differed among several strains, suggesting that class II molecules of different MHC haplotypes may preferentially use different sets of proteases for degrading Ii.

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Intracellular transport and function of H2-M

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MHC class II molecules acquire peptides in the endosomal/lysosmal system and HLA-DM has been shown to be important for this process in human B cell lines. In contrast to conventional class II molecules, HLA-DM and the equivalent murine molecule, H2-M, are localized intracellularly in lysosome-like compartments and a tyrosine-based signal in the cytoplasmic tail of the β chain is sufficient to deliver H2-M to lysosomes.

HLA-DM facilitates removal of class II-associated invariant chain peptides in vitro, but the in vivo relevance of this reaction has been uncertain. To study the function of H2-M in vivo, we generated mice lacking H2-Ma and thus functional H2-M. These mice have normal levels of class II at the cell surface, but the vast majority of class II molecules are associated with invariant chain-derived CLIP peptides and appear to be conformationally distinct. In H2-M-deficient mice significant numbers of $CD4^+$ T cells develop, indicating that positive selection occurs in the thymus. The $CD4^+$ cells are unresponsive to self H2-M-deficient antigen presenting cells (APCs), but are hyperreactive to APCs from wild-type littermates and, to a lesser extent, MHC-allogeneic APCs. The H2-M-deficient APCs fail to elicit proliferative responses of either wild-type MHC-syngeneic T cells or MHC-allogeneic T cells.

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Partho Ghosh, Miguel Amaya, Elizabeth Mellins, Don C. Wiley

"Implications of the structure for antigen loading on class II MHC"

A complex between HLA-DR3 and a fragment of invariant chain called CLIP was isolated from a human cell line defective in antigen presentation and its X-ray crystal structure has been determined from crystals grown at the pH (4.5) of endosomes. Previous data indicate that this complex is an intermediate in class II histocompatibility maturation, occurring between invariant chain-DR3 and antigenic peptide-DR3 complexes. The structure reveals that CLIP occupies the peptide binding site and binds almost identically to the way a peptide antigen, influenza virus haemagglutinin (HA 306-318), binds to DR1. The contacts between DR3 and CLIP suggest that the CLIP segment of Ii would stabilize most class II molecules almost as much as an antigenic peptide. We measured the half-life of CLIP bound to DR3 (37° C, pH 4.5) to be almost 4 hours, long enough to require an exchange process, such as that mediated by HLA-DM, to remove CLIP from most subtypes of class II MHC before antigenic peptides can bind *in vivo*. The conformation of DR3 in the DR3-CLIP complex is only slightly different from DR1 in DR1-HA, though enough to explain antigenic differences without recourse to allosteric transitions or novel conformations. Evidence is presented that the structure observed here is the substrate for the HLA-DM exchanger, limiting the possible mechanisms for the catalytic activity of HLA-DM.

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2.- Presentation of endogenous antigens by MHC class II

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The role of invariant chain and H-2M in class II restricted antigen presentation.

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Our laboratory has studied the role of the protein co-factors invariant chain (Ii) and DM in presentation of antigen by murine class II molecules in which a transfection model has been implemented to introduce class II molecules into antigen presenting cells (APC). Genes encoding the protein co-factors DM and Ii have then been introduced by supertransfection of the class II positive cells. Biochemical and functional analysis of the derived transfectants have led to several general conclusions. For I-A^d, we have found that the majority of T cell epitopes derived from exogenous antigens are generated as efficiently in the absence of invariant chain as in its presence. The presence of DM within the APC similarly does not enhance antigen presentation. Several T cells specific for exogenous antigens display enhanced reactivity with an invariant chain positive, DM negative antigen presenting cell compared to cells expressing class II alone. The further contribution of DM in these responses is variable, and ranges from 4-100 fold further shifts in the antigen dose response. Thus the majority of responses to exogenous antigens occur independently of the Ii and DM protein co-factors: they occasionally enhance but are not essential for generation of the peptide class II complex.

In contrast to the results obtained with exogenous antigens, we find striking effects of DM and Ii on endogenous antigen presentation, using alloreactive T cells as the primary readout. A panel of 35 anti-I-A^d alloreactive T cells have been examined for their dependency on Ii and DM for recognition. We have found that, unlike the exogenous antigen specific T cells, only a minority (3) are indifferent to the presence of Ii and DM. The vast majority of epitopes recognized by alloreactive T cells are affected by the presence of Ii and DM within the APC. Of particular surprise were the results obtained when DM effects were examined. Here, of the approximately 30 T cells whose reactivity was affected by Ii, two were blocked by Ii and DM, either alone or together, approximately half were enhanced by Ii and were further enhanced by the presence of DM within the APC. Unexpectedly, approximately half of the T cells that required Ii for recognition were antagonized by the presence of DM.

Thus, our data suggest that effects of DM can be either positive or negative, and point to the function of this molecule as peptide exchange protein, that can either increase or decrease expression of particular peptide class II complex. The effects of DM on antigen presentation are thus unpredictable and clearly extend beyond removal of CLIP. The effects depend on the allele of class II molecule studied and the antigen that is used as the source of peptide. Epitopes generated from exogenous antigens in an experimental setting are likely to be more independent of the participation of the protein co-factors, whereas epitopes derived from the internal antigens that constitutively occupy the class II binding pocket appear to be most dramatically affected by the presence of DM within the APC.

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3.- Professional and non professional APCs

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RELATIVE ROLE OF DENDRITIC AND B CELLS IN ANTIGEN PRESENTATION TO MHC CLASS II-RESTRICTED T CELLS IN VIVO

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Both self and non-self antigens, either of endogenous or exogenous origin, are processed and presented in vivo by MHC class II molecules. Naturally processed self β2 microglobulin (β2M) epitopes are constitutively presented to CD4+ T cells by any class II-positive antigen-presenting cell (APC) tested, but with highest efficiency by splenic and thymic DC, followed by macrophages, large B cells, and small B cells (1). This hierarchy of self β2M presentation does not depend on differential processing capacity of these APC populations, and it correlates with expression of CTLA-4 ligands and ICAM-1 molecules, rather than with expression of class II molecules (2).

To study presentation of exogenous antigens, draining lymph node cells (LNC) from mice immunized subcutaneously with hen egg-white lysozyme (HEL) in adjuvant were used to stimulate, in the absence of any further antigen addition, HEL peptide-specific T hybridoma cells. The antigen-presenting capacity of three different APC populations recruited in lymph nodes, DC (N418+, class II+, B220-, low buoyant density), large B cells (B220+, low buoyant density) and small B cells (B220+, high buoyant density), was analysed using this read-out system. Following immunization with HEL in adjuvant, DC are the only lymph node APC population expressing detectable HEL peptide-class II complexes. These results indicate that lymph node DC and not B cells are the APC population initiating the immune response in vivo following administration of antigen in adjuvant (3).

Conversely, after i.v. administration of soluble HEL in mice previously injected with adjuvant only, lymph node B cells are much more efficient than DC in presenting antigenic complexes to T cells. These data correlate with the observation that, unlike B cells, lymph node DC present in vitro to class II-restricted T cells protein antigen much less efficiently than synthetic peptides, probably as a consequence of their maturation process (4).

IL-12 is a key regulatory cytokine produced by APC which drives the development of interferon-γ (IFN-γ)-producing cells and promotes cell-mediated immunity. We have analysed secretion of bioactive IL-12 p75 by cell populations enriched in dendritic cells (DC), macrophages or B cells. These APC populations do not produce IL-12 constitutively but, upon stimulation with heat fixed *Staphylococcus aureus* and IFN-γ, IL-12 is secreted by DC and macrophages, whereas B cells fail to produce it. Co-culture with CD4+ T cell hybridomas induce a low but detectable IL-12 production by DC and macrophages, but not by B cells. The interaction between APC and T cells leading to IL-12 production is mediated by CD40-CD40L molecules. IL-12 secretion by DC is highly increased by antigen-specific interaction with CD4+ cells, whereas IL-12 secretion by macrophages is only slightly upregulated. Even under these conditions B

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cells fail to produce IL-12. These results indicate that interaction with antigen-specific T cells can increase IL-12 production by DC, maximally by lymph node as compared to splenic DC. Upregulation of IL-12 secretion by interaction with antigen-specific T cells is abrogated by soluble CD40 and by anti-class II mAb, demonstrating a positive feedback between T cells and DC mediated by CD40-CD40L and TCR-peptide/class II interactions. Following subcutaneous immunization with protein antigen in adjuvant DC are the only APC expressing antigenic complexes and secreting, upon interaction with antigen-specific T cells, substantial amounts of IL-12. The capacity of lymph node DC to present efficiently antigenic peptides and to secrete IL-12 could explain their unique capacity to promote proliferation and differentiation of naive $CD4^+$ T cells in vivo.

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EPITHELIAL CELLS VERSUS PROFESSIONAL APC IN AUTOANTIGEN PRESENTATION: AN EXPERIMENTAL MODEL.

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T cell responses in autoimmunity are the key to interpret the mechanisms leading to tissue damage and of their regulation. However, their study has been impaired for a long time by the difficulties to obtain good antigen presenting cells (APC). The question of whether the autoantigen recognition by T cells is a consequence of the presentation of tissue debris by macrophages and other professional APCs in situ or comes from recognition of autoantigen expressed by the autologous epithelial cells is an open one. The answer could be important since different cell types may be capable of presenting a different set of peptides from the same antigen and therefore induce differential responses in T cells. We have designed a model by which we can compare autoantigen presentation by professional (B cells) and non professional (epithelial cells) APCs to specific T cells.

Professional APCs were obtained by the transfection of FcγRI (CD64) on EBV transformed B cells allowing them to bind internalize and process antibody-antigen complexes for presentation to specific T cells. This model has been established using a cytotoxic CD4+ T cell clone specific for influenza H3 peptide 307-318 (13D) restricted by HLA-DR1 and a DR1+ CD64+ positive monocytic cell line THP1 as APC.

Target cells in organ specific autoimmunity are generally epithelial cells which express organ-specific autoantigens, such as thyroid follicular cells or beta cells. These cells express class II molecules, not normally expressed and presumably induced by the cytokines present in the microenvironement. They also express Ii and possibly HLA-DM, although it is not clear whether this expression is enough for an efficient transport and peptide exchange and therefore if they are capable of presenting antigen to autoreactive T cells. As a model of autoantigen presentation by epithelial cells, we have used an insulinoma cell line, RIN-B, which expresses most of the putative IDDM autoantigens as the basis to construct a panel of transfectants expressing different combinations of HLA-DR4, human B7 as a co-stimulatory molecule and the invariant chain (Ii) and HLA-DM so the class II molecules expressed are internally transported and loaded with peptide in a way similar to a normal APC.

In a first screening, the presence of HLA-DR in addition to Ii and B7 but in the absence of DM, makes an epithelial cell capable of inducing a primary response in PBL whereas HLA-DR alone was enough to stimulate allo-specific cell lines. These data are now been completed with a series of clones both allogeneic and antigen specific, allowing the comparison of antigen presentation by both types of APCs. In addition, the processing capacity of the cells will also be asessed by the analysis of peptides bound to HLA-DR4 in the different transfectants.

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C. Functional role of MHC class II in a normal and abnormal immune response

Chairpersons: Laurie H. Glimcher and Richard A. Flavell

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1.- Role of MHC class II in T cell ontogeny and tolerance

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THE ROLE OF CIITA IN MHC BIOLOGY REVEALED BY GENE TARGETING

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The Major Histocompatibility Complex Class II Transactivator (CIITA) is believed to be required for both constitutive and IFN–γ inducible expression of MHC class II genes (Steimle et al., 1993; Chang et al., 1994; Steimle et al., 1994). In addition, introduction of the CIITA gene driven by a constitutive promoter is sufficient to activate MHC class II genes in plasmacytoma cells or mouse T cells where MHC class II genes are not normally expressed (Silacci et al., 1994; Chang et al., 1995). Furthermore, in cell lines CIITA regulates the expression of HLA-DM and invariant chain (Ii) genes which are involved in antigen presentation (Chang and Flavell, 1995; Chin et al., 1995; Kern et al., 1995).

To understand the role of CIITA in vivo, we have used gene targeting to generate mice that lack CIITA. CIITA deficient (-/-) mice do not express conventional MHC class II molecules on the surface of splenic B cells and dendritic cells. In addition, macrophages resident in the peritoneal cavity do not express MHC class II molecules upon IFN–γ stimulation nor do somatic tissues of mice Injected with IFN-γ in contrast to wild type mice. CIITA is also required for maximal expression of Ii and H-2M genes in B cells because the levels of these gene transcripts are substantially decreased but not absent in CIITA (-/-) mice. The transcription of non conventional MHC class II genes is, however, not affected by CIITA deficiency. CIITA, therefore, plays a critical role in both constitutive and IFN–γ inducible expression of conventional MHC class II genes in vivo. However, a subset of thymic epithelial cells express MHC class II molecules. Nonetheless, very few mature CD4 T cells are present in the periphery of CIITA (-/-) mice despite MHC class II expression in the thymus. As a consequence, CIITA (-/-) mice are impaired in T-dependent antigen responses and MHC class II mediated allogeneic reponses. CIITA (-/-) mice can be utilized as a model system for human BLS type II.

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Unopposed Positive Selection and Autoreactivity in Mice Expressing Class II MHC Only on Thymic Cortex

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Positive and negative selection play an important role in the development of the T cell repertoire and the acquisition of self-tolerance. However, the precise anatomic sites and temporal relationship of these events has been unclear. We have used the human keratin 14 promoter to re-express an Abb cDNA in I-Ab null mice. The transgenic I-A molecule is expressed only in thymic cortical epithelium: thymic medullary epithelium and bone marrow-derived cells are I-A negative. Superantigen-mediated clonal deletion does not occur in the "K14" mice or in relB(-/-) mice which lack a thymic medulla. In the"K14" mice, CD4+ cells are positively selected. However, these cells are autoreactive: they proliferate extensively to and specifically lyse I-Ab positive target cells. These autoreactive cells comprise 5% of peripheral CD4 T cells providing a minimal frequency of cells that must normally undergo negative selection to preserve self-tolerance. These "K14" mice demonstrate that positive selection is an anatomically and temporally distinct process from negative selection.

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THYMIC SELECTION AND PERIPHERAL HOMEOSTASIS OF SUPERANTIGEN-REACTIVE T CELLS

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Superantigens (Sag) are products of bacterial or viral origin that specifically bind to MHC class II molecules and interact with the Vβ domain of the T cell receptor. During T cell development immature thymocytes that encounter SAg:MHC complexes are clonally deleted by a mechanism known as apoptosis. In contrast the response of mature T lymphocytes to Sag is very complex and involves proliferation, apoptosis and the induction of anergy.

We have used the endogenous Sag encoded by mouse mammary tumor virus[1] in order to study the cellular requirements for clonal deletion of autoreactive T lymphocytes. Our data indicate that self-SAg reactive thymocytes are deleted relatively late in development (as they migrate from the cortex to the medulla), probably due to encounter with Sag-expressing dendritic cells present in the cortico-medullary junction.

In addition to thymic selection, we have investigated in detail the fate of mature T cells activated by the bacterial Sag Staphylococcal enterotoxin B (SEB) *in vivo*. Our data demonstrate that most proliferating SEB-reactive T cells eventually undergo apoptosis[2]. Furthermore, apoptosis of proliferating T cells is dependent upon an intact Fas pathway, since it is severely compromised in *gld* or *lpr* mice that have spontaneous mutations in the Fas ligand and Fas receptor, respectively. More recent studies[3] implicate a $B220^+$ T cell blast expressing Fas ligand as the putative precursor of SEB-induced apoptotic T cells. Interestingly, T cells with a similar phenotype can be found in the enlarged lymph nodes of unimmunized *lpr* and *gld* mice, where (in the absence of Fas-mediated cell death) they apparently give rise to the accumulating $CD4^-$ $CD8^-$ $B220^+$ T cell population.

Collectively our data indicate that Sags are a useful tool to study thymic selection and peripheral homeostasis of T cells.

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2.- MHC class II and Diseases

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HUMAN T CELL DEVELOPMENT: LESSONS FROM PATIENTS LACKING MHC CLASS II EXPRESSION.

Peter van den Elsen, Judy Henwood, Anette van Boxel-Dezaire, Ron Schipper, Martijn den Hoedt, Ad Peijnenburg, Özden Sanal, Fügen Ersoy, Ger Rijkers, Ben Zegers, Jaak Vossen, Maarten van Tol and Gail Hawes.

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Patients with an MHC class II deficiency (bare lymphocyte syndrome) provide an unique opportunity to study development of the human T cell receptor repertoire. The lack of MHC class II antigen expression in these patients resulted in a strong reduction in the numbers of $CD4^{+}CD8^{-}$ T cells in the circulation. These peripheral $CD4^{+}CD8^{-}$ T cells were not restricted in the employment of TCR V- and J-gene segments but the expressed TCRs manifested different CDR3 amino acid incorporation profiles resulting in altered charge and hydropathicity properties of the presumed antigen binding site. Moreover, the lack of MHC class II antigen expression had altered the $CD4^{+}CD8^{-}$skewing patterns of TCR V-gene families which can be found in normals. In conclusion, the lack of MHC class II antigen expression had a profound impact on the $CD4^{+}CD8^{-}$ TCR V-gene skewing patterns and on the amino acid profiles of the CDR3 in $CD4^{+}CD8^{-}$ T cells isolated from MHC class II-deficient patients. The altered amino acid profiles resulted in significant changes to the local environment of those CDR3s, which would have implications for antigen binding, and may reflect T cells that have escaped the normal selection processes in the thymus, where the affinity and avidity of the TCR interaction with MHC are thought to dictate those selection processes.

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CHARACTERIZATION OF HUMAN NICKEL-SPECIFIC T CELL CLONES.

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Contact dermatitis is a skin disease involving the proliferation of T cells in response to low molecular weight compounds. Heavy metal ions are well known contact sensitizers and the most common in humans is nickel with about 10% of caucasian population sensitized. A notable proportion of nickel-allergic patients are also reactive to other metals such as copper, cobalt and palladium. Our purpose was to characterize the T cells involved in that disease and to investigate whether allergic reactions to different haptens in one donor result from independent co-sensitizations to these metals or might be cross-reactions due to the capacity of some T cells to equally recognize the different metal ions. Hapten-specific T cell lines were produced by in vitro stimulation of peripheral blood mononuclear cells from four nickel-allergic donors with $NiSO_4$. Phenotypical analysis of these T cell lines indicated a majority (80-90%) of CD4+ T cells expressing an $\alpha\beta$ TcR. A panel of 42 different T cell clones was then isolated. Again, the majority of the T cell clones obtained belonged to the CD4+ subset. They proliferated to Ni in the presence of autologous EBV-B cells in a MHC class II restricted way (either DP, DR or DQ). Using fixed autologous APC, we could distinguish two distinct groups of T cell clones on the basis of processing requirements : 40% of the T cell clones were strictly processing-dependent, whereas the remaining 60% could proliferate in response to Ni even in the presence of glutaraldehyde-fixed APC. The capacity of these T cell clones to react to other metals (Cr, Co, Cu, Pd, Pt and Zn) was tested using autologous EBV-B cells as APC. The majority of the Ni-specific T cell clones did proliferate to either Cu or Pd. By contrast, none of them proliferated to either Zn (not reported as contact sensitizer) or to Cr and Pt (usually not associated to nickel allergy). A marginal cross-reactivity to Co was observed with one T cell clone also recognizing both Cu and Pd. These results indicate the capacity of most of the Ni-specific T cell clones to recognize *in vitro* either Cu or Pd, which suggest that, *in vivo*, allergic reactions to Cu and Pd observed in Ni-sensitive patients may be due to cross-reactivities at the clonal level. The aim of our further study will be to characterize the nature of the epitopes which are recognized by these metal-specific T cell clones and different models may be postulated: a complex of Ni with soluble or surface proteins which, upon processing, might be presented as Ni-peptide-complexes, a direct binding of Ni to peptides already associated with class II MHC molecules on the APC. The coordination of Ni with amino acid side chains of MHC class II molecules themselves, as well as the effect of Ni salts on the processing of self proteins, allowing the generation of neo-self determinants, might also be envisaged.

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POSTERS

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ANALYSIS OF ANTIGENIC PEPTIDES PRESENTED BY MHC CLASS II MOLECULES ON THE SURFACE OF THE T CELL LEUKEMIC CELL LINE, H9

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During thymic education, thymocytes are positively and negatively selected within the thymus to recognize antigens in the context of MHC molecules in order to initiate proper immune reactions. We have previously reported (1) that the MHC Class II antigen, DR, is expressed on human thymocytes, suggesting the possibility that DR-positive thymocytes may play an important role as antigen presenting cells (APC) which participate in the positive selection process. To demonstrate this possibility, we selected the H9 T cell leukemic cell line expressing DR molecules and tested them to see whether they could function as APC. The genotype of these cells was DRβ1 0403 and DRβ4 0101 and the HLA-DR molecule from this cell line was purified through an affinity columns and the peptides were eluted. Twelve kinds of peptides were fractionated and the size of eluted peptides varied from 8 to 19 residues. Five of them had the conserved residues, proline and valine, at the relative positions of +1 and +6, which are considered to be found at the anchor site of the DR groove. Interestingly, two of the peptides were thought to come from their own HLA antigens and HMG-2. Furthermore, fraction 26 was found to be a peptide fragment derived from the nonpolymorphic region of the HLA α1 domain and is thought to have originated from HLA-A, B, or C of H9. Fraction 39 matched 5-17 residues of the HMG-2 protein, which is known to be involved in T cell differentiation and development. These data demonstrate that the human MHC Class II molecule, DR, expressed on T cell leukemic cells is just as capable of presenting endogenously derived peptides as professional APC. Taken together, this report shows that DR positive T cells are able to present endogenous peptides in the DR groove and supports the idea that human thymocytes expressing DR molecules may be involved in thymic education.

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1) Human Immunol. 33, 294-298 (1992)

Instituto Juan March (Madrid)

Mice deficient in MHC class II transactivator (CIITA) show tissue specific impairment. Cheong-Hee Chang, Sylvie Guerder, Soon-Cheol Hong, Richard Flavell. Section of Immunobiology, Yale School of Medicine, 310 Cedar Street, New Haven, CT 06510.

CIITA is a transcriptional factor and is required for both constitutive and IFN-γ inducible expression of class II genes (1-3). In addition, CIITA activates not only MHC class II genes but also genes involved in antigen presentation, such as the invariant chain (Ii) and H-2M genes (4, 5). Furthermore, the introduction of CIITA into MHC class II negative cells, plasmacytoma cells and mouse T cells, is sufficient to activate these genes (6, 7). The role of CIITA in vivo has further been characterized by producing mice deficient in the expression of CIITA gene, CIITA (-/-), by homologous recombination.

The phenotype of CIITA (-/-) mice is as follows: (1) Both I-A and I-E MHC class II molecules are absent on antigen presenting cells. (2) IFN-γ inducible expression of MHC class II is impaired in these mice. (3) A subset of epithelial cells in the thymus of CIITA (-/-) mice express low levels of MHC class II molecules. (4) CD4 T cells are not present in the periphery of CIITA (-/-) mice despite the presence of these MHC class II expressing cells in the thymus. Cell transfer study show that the lack of mature CD4 T cells in the periphery is a result of defective thymic microenvironment, not an intrinsic defect of CD4 T cells. (5) The levels of Ii and H-2M gene transcripts are greatly reduced in CIITA (-/-) mice. (6) The absence of MHC class II molecules in CIITA (-/-) mice is uniformly observed, regardless of haplotype of H-2 locus.

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Translational control of TNFα expression by MHC class II ligands

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The stimulation of human monocytes with superantigens induces the expression of TNFα. We have measured the TNFα mRNA accumulation and the rate of TNFα biosynthesis.
The induction at the transcriptional level reaches a maximum 1 h after the stimulus. The biosynthesis rate of metabolically labeled TNFα is higher than the accumulation of its mRNA. The polysome gradients prepared with monocytes stimulated with superantigen show an increase in the TNFα mRNA bound to the polyribosome fractions, compared to non-stimulated cells.
These data suggest a translational control of TNFα mRNA by MHC class II ligands.

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THE EXPRESSION OF IAß IS REGULATED AT THE LEVEL OF TRANSLATION

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Macrophages play a key role in regulating the immune response, by presenting antigens bound to MHC class II molecules to T cells. IFNγ is known as the major cytokine responsible of macrophage activation as well as the main MHC class II gene inducer in these cells. After 48 h of IFNγ stimulation, the expression of both IAα and IAß mRNA reached a maximal level. At this time the increase of mRNA for IAß was 25-fold but the protein synthesis rate of IAß increased 50-fold, which suggests a translational control. In order to assess the possibility of a translational induction we analyzed polysome gradients from non stimulated macrophages and 48 h-IFNγ-treated macrophages. Our data show that, upon IFNγ stimulation, there is a marked shift in the polysome gradient profile of both IAα and IAß mRNA toward the polysome bound fractions. However, ß-actin mRNA, used as a control, did not change its polysome distribution profile. These data demonstrate an increased efficiency in translation initiation of both chains of IA. Therefore, we conclude that upon IFNγ stimulation of macrophages the expression of IA is regulated at the level of translation.

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EPITHELIAL CELLS AS APC: REQUIREMENTS FOR INDUCTION AND MAINTENANCE OF T CELL RESPONSES

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We have used an insulinoma cell line, RIN-B, which expresses most of the putative IDDM autoantigens (GAD 65 and 67, insulin, CPH, 38 kDa insulin granule protein). ICA positive sera from IDDM patients are positive with RIN-B cells and it has been shown that T cells from IDDM patients have specific reactivity to RIN-B extracts which can specifically induce the proliferation of a T cell clone specific for the 38kDa of islet granules.

Our aim is to study the capacity and the requirements of an endocrine epithelial cell to process and present target autoantigens to T cells. We are using the RIN cell line as the basis to construct a panel of transfectants expressing different combinations of HLA molecules DR4 or DR3, human B7 as a co-stimulatory molecule and the invariant chain (Ii) and HLA-DM so the class II molecules expressed are internally transported and loaded with peptide in a way similar to a normal APC. This panel of transfectants is being used to study their capacity:

1- to induce a primary allogeneic response from PBL

2- to activate the response of alloreactive, antigen specific and autoreactive T cell clones.

In a first screening, the presence of HLA-DR in addition to Ii and B7 but in the absence of DM, makes an epithelial cell capable of inducing a primary response in PBL. The different combinations are now being tested to precise which are the requirements for a primary response induction. On the other hand, the first screening also suggested that HLA-DR alone was enough to stimulate allo-specific cell lines. These data are now been completed with a series of clones both allogeneic and antigen specific.

The processing capacity of the cells will be asessed in the future by the analysis of peptides bound to HLA-DR4 or DR3 in the different transfectants.

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HLA-DM - a Molecular Chaperone and Catalyst for Peptide Loading of MHC class II Molecules

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HLA-DM (DM) is a non-classical major histocompatibility complex (MHC) class II molecule that has recently been shown to facilitate peptide loading of classical MHC class II molecules at endosomal pH. This effect is accomplished by accelerating the release of CLIP peptides which are residual fragments of the proteolytic destruction of the invariant chain in endosomal/lysosomal compartments. We provide evidence that DM also affects the loading process per se in addition to its CLIP removal function: (i) after CLIP release DM protects empty DR αß dimers from aggregation via formation of intermediary DR-DM complexes at the same time allowing rapid and stable loading of DR molecules, (ii) the DM catalysis of loading follows Michaelis-Menten kinetics attaining turnover numbers of 3 - 12 molecules per minute depending on the allele and the peptide. Thus, DM acts as a true molecular chaperone following enzyme-like kinetics.

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GENE THERAPY IN CIITA DEFICIENT BARE LYMPHOCYTE SYNDROME

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The defective gene for one complementation group of HLA class II immunodeficiency or Bare Lymphocyte Syndrome has been identified as the transactivator, CIITA. Using recombinant retroviruses, we hope to activate class II expression in the appropriate cells and to establish the feasibility of inserting and expressing the vector in peripheral lymphocytes and/or bone marrow derived cells from BLS patients. We have generated cell lines that produce CIITA containing viruses in a modified vector and developed methods for highly efficient transduction of the virus in cell line. We compared the efficiencies of transduction using different methods with the cell line BLS-2. The infected cells were stained with antibodies against HLA-DR and analyzed by flow cytometry. The greatest efficiency was achieved by irradiating the virus producer cells and then co-cultivating them for 12 to 24 hr with the target cells. The efficiency of transduction by co-cultivation has been in the range of 20 to 30% 72 hr post infection. Although we hoped to achieve tissue specificity using the class II associated invariant chain (Ii) promoter, we have observed that all class II negative cell lines the we have infected with this vector, can be induced to express class II. Furthermore, we have evidence that leaky expression of CIITA from either promoter can up-regulate the Ii promoter and drive high levels of class II expression.

PBMCs from a BLS patient were infected with the vector, and subsequently cultured in the presence of IL-2 and PHA. As we expected, three days after infection most of the cells growing out in this system were T cells, and approximately 6% were positive for HLA-DR. A few were positive for the CD20 B cel l marker, but of these about half were also positive for HLA-DR, suggesting that B cells might be preferentially transduced. CD34+ cells were obtained and cocultured with the producers in the presence of FCS and cytokines. Following coculture, the expanding cells were harvested and recultured with c-*kit* ligand, GM-CSF, and TNFα. The myeloid progeny were successfully transduced, based on HLA-DR expression by 29% of the bulk population. Moreover, among the population expressing CD1a after one week of expansion, 37% of these CD1a positive dendritic/Langerhans cells expressed HLA-DR compared to the control.

In order to assess stability *in vivo*, we injected SCID mice with transduced and untransduced B cells from BLS patients. The tumors were established over the course of 12 weeks. There was a small increase in the growth rate of tumors from transduced cells. The transduced BLS cells that were uniformly positive in culture remained at least 85% positive after the lymphoma developed, and a line that had a low percentage of positive cells increased its expression dramatically. These results confirm our prediction that expression should be very stable and also suggest that the CIITA transduced cells may have a competitive advantage *in vivo*. Indeed, if there is a competitive advantage for the CIITA transduced bone marrow progenitors in the BLS patient, then engraftment of the transduced cells will be selected after introduction.

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HLA-DO IS A LYSOSOMAL RESIDENT WHICH REQUIRES HLA-DM FOR ITS INTRACELLULAR TRANSPORT

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Abstract

The murine MHC class II molecule H2-O is expressed in B cells and in thymic epithelium but the human equivalent, HLA-DO (DO) has not been detected, though the corresponding genes, *HLA-DNA* and *HLA-DOB*, are well known. Here we show DO to be a lysosomal resident in B cells. Surprisingly, DO forms stable complexes with HLA-DM (DM), another lysosomal class II-like molecule which is important for class II-restricted antigen presentation. Association with DM is necessary for efficient exit of DO from the endoplasmic reticulum (ER) and for accumulation in lysosomes. The association is evolutionary conserved and in mice lacking H2-M, the mouse equivalent of DM, H2-O is retained in the ER. The DO-DM complexes survive in the lysosomal system suggesting that DO and DM functions may be intertwined.

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INTERNALIZATION OF IMMUNOCOMPLEXES Ab-Ag AND ANTIGEN PRESENTATION IS IMPROVED ON LCLs AFTER TRANSFECTION OF HIGH AFFINITY FcgRI. AN EXPERIMENTAL MODEL TO STUDY PROCESSING AND PRESENTATION OF AUTOANTIGENS IN AUTOIMMUNITY.

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The study of T cell response in diabetic patients has been impaired for a long time by the difficulties to generate a good antigen presenting cell (APC). The advantage of autologous EBV cell lines from each patient has a handicap: B cells are good as APC only when the Ag is internalized trough its specific membrane immunoglobulin (BCR) and this is difficult to obtain. Nowadays there are some good candidates as autoantigen targets in Insulin-Dependent Diabetes Mellitus (IDDM), but their isolation and purification is not easy. Human islet extracts are considered as the best source of autoantigen but LCL lines are not good in the internalization and processing of these complex extracts.

In order to bypass this problem, we have designed a model that consist in the transfection of FcgRI (CD64) on EBV transformed B cells allowing them to take profit of a physiologic strategy used by all FcR positive cells: an antibody with a known specificity binds to FcR acting as a link between the antigen and the APC (the autologous B cell) and allowing internalization of the antigen.

As a read-out for this model, we have compared the level of recognition of antigen by influenza hemmaglutinin peptide (H3 307 - 318)-specific and HLA-DR1 restricted T cell clones. Purified antigen (H3) and of a preparation of UV-inactivated influenza virus extract (UV-flu) were used in the presence or absence of an anti-H3 Ab. As APC we have used a DR1-positive human monocytic cell line (THP-1) which expresses high levels of CD64 upon stimulation with IFN-g, as tested by cytoflurometry. Briefly, THP-1 cells where washed after o/n stimulation with IFN-g, and labelled with 51Cr; samples were incubated for 30 min at 4° C with a policlonal Ab anti-H3 (a-H3), washed and incubated in the same H3 protein or UV-flu. After washing they were incubated for 1 h at 37 C for internalization, while untreated samples were kept on ice, and pulsed with the antigen for the last hour at 37 C. H3 peptide was used as a positive control. Our data shows that % of lysis increased 2-3 fold when the antigen is internalized via CD64 compared with the absence of a-H3. The capacity of transfected FcRs to internalize Ab-Ag complexes has been demonstrated in an epithelial cell line and now a homozigous DR1- positive LCL line (HOM-2) has been transfected with CD64 to compare results.

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STUDY OF THE MOUSE MHC CLASS II TRANSACTIVATOR :

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Major Histocompatibility Complex (MHC) class II gene expression is tissue specific and controlled mainly at the transcritional level. By complementation cloning, we isolated a human gene (CIITA) coding for an MHC Class II transactivator shown to be required for the expression of MHC class II genes in both B cells and interferon gamma inducible cells. Morever CIITA is known to be able to activate MHC class II genes but also genes required for antigen presentation.

We isolated the mouse CIITA gene by hybridisation screening of a mouse cDNA library. By mouse genomic Southern study, the cloned cDNA was shown to be the closest homologue of the human CIITA. Both the human and the mouse genes show the same differential expression correlated with the MHC class II expression. Morever mouse CIITA expression mRNA levels coincide with MHC class II expression quantitatively in multiple mouse tissues. The putative quantitative and causal relationship between the CIITA mRNA level and the MHC class II levels is being currently tested.

Transfections of both the human and the murine CIITA induce similar level of MHC class II expression in the mouse cell line LMTK. Both the human and the murine CIITA are able to correct the CIITA deficient human cell line RJ2.25. Morever both genes have the ability to activate the same set of genes. The mouse and human CIITA gene are thus functionally equivalent.

In summary the mouse homologue CIITA has been isolated and should allow a number of studies dealing with the effects of this gene and the regulation of MHC class II genes both in vitro and in vivo.

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The tetramer model: a new view of class II major histocompatibility complex molecules and antigenic presentetation to CD4 T cells.

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Major histocompatibility complex class II molecules bind antigenic peptides forming complexes that interact with T cell receptors on CD4+ lymphocytes triggering immune responses. We present here a model which includes a new view of major histocompatibility complex class II molecules along their pathway to the cell surface and a qualitative model for its interaction with T cell receptor. Based on the idea that preformed class II tetramers on the antigen presenting cell surface have two peptide binding sites, we offer a molecular model in which class II tetramers are able to deliver qualitatively different signals to T cells. We propose that T cell receptor engagement could lead to full activation if class II tetrtamers are loaded with two identical peptides, while tetramers bearing two different peptides would commit T cells to an anergic status. So, tetrameric class II complex could be viewed as a quantitative-qualitative transducer that would trigger either an immune response or anergic status depending on the concentration of antigenic peptides. We also propose that, at the thymic level, tetrameric class II molecules with two different peptides would signal for positive selection and tetramers with identical peptides would trigger negative selection.

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HERPESVIRUS SAIMIRI IMMORTALIZATION OF HUMAN T CELLS LACKING HLA CLASS II MOLECULES

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Besides their role in antigen presentation, HLA class II molecules have been shown to mediate signal transduction in B cells as well as in synovial and endothelial cells. Several responses have been measured, including proliferation, homotypic aggregation, immunoglobulin production, and apoptosis. In order to investigate the possible functional role of HLA class II molecules on the surface of T lymphocytes (they are induced on these cells upon activation), we have immortalized T cells from a patient lacking HLA class II with *Herpesvirus saimiri* and compared them with normal controls. Normal T cells transformed with this virus express HLA class II constitutively and are a good model to study their physicpathology, since both TCR/CD3-mediated signals and HLA-mediated signals are preserved. HLA-DP, DQ and DR, were selectively absent on the patients cells by phenotypical (Moab staining: 1%, 5%, 1% respectively as compared to 99%, 96%, 100% in controls) and functional criteria (Ca^{2+} flux via HLA-DP). The cells were CD8+ and TCR/CD3-mediated signals were preserved (Ca^{2+} flux). Thus, *Herpesvirus saimiri*-immortalized T cells lacking HLA class II molecules are a good model to study the role and regulation of MHC class II expression in human T cells.

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Antigen Specific T Cells Responsiveness in the Absence of Invariant Chain.

Idit Shachar, Iqbal S. Grewal, Dan Zekzer and Richard A. Flavell. Section of Immunobiology HHMI. Yale University School of Medicine, New-Haven, CT. 06510.

MHC class II molecules associate with invariant chain (Ii) during biosynthesis. Ii facilitates folding of class II molecules, interferes with their association with peptides, and is involved in their transport. Cells form invariant chain deficient mice (ΔIi) show aberrant transport of MHC class II molecules, resulting in reduced levels of class II complexes at the surface. Deletion of the Ii gene greatly diminishes the ability of splenic B-cells to present exogenous protein antigen *in vitro* and *in vivo*. Furthermore, mice lacking the Ii chain have reduced numbers of CD4+ T-cells and elevated CD8+ population in the periphery. However, low levels of the p31 and p41 invariant chain isoforms reconstitute the CD4+ population and antigen presentation.
To determine the CD4+ T-cells responsiveness in the absence of the invariant chain and in the presence of the two isoforms p31 and p41, we immunized mice with the protein antigen key hole limpet hemocyanin (KLH) in CFA. Nine days later bulk draining lymph nodes recall response to KLH was measured from draining lymph nodes. Unfractionated total LN cells from ΔIi mice responded poorly to KLH whereas the wild type and p31 and p41 cells showed a good recall proliferative response. However, enriched CD4+ T-cells from draining lymph nodes from ΔIi mice responded even better than the wild type cells. These results indicate that despite the low levels of CD4+ T-cells in the ΔIi mice responsiveness to protein antigen is preserved. The implications of these results will be discussed.

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IDENTIFICATION AND REGULATION OF IAX: AN MHC CLASS II TRANSCRIPTION FACTOR

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Expression of Major histocompatibility complex (MHC) class II molecules is controlled by many *cis* and *trans* elements. Furthermore, expression of MHC class II genes is cell specific and can be regulated by several cytokines, such as interferon γ.

IAX has been identified as a transcription factor that binds to the MHC class II IA_β X box. The binding site corresponds to the X1 (5') site of the X box. Antisense constructs of the IAX gene were shown to lower the level of expression from the IA_β promoter.

The IAX gene codes for a protein of about 36 kD, and two bands are detected by Western blot analysis. In mouse, IAX mRNA and protein are present in B cells and macrophages, but are undetectable in T cells and fibroblasts. Furthermore, Interferon γ induces IAX mRNA and protein expression in macrophages and fibroblast cells. Thus, the pattern of expression and regulation of IAX gene is similar to modulation of MHC class II gene expression. On the other hand, induction of IAX mRNA precedes expression of MHC class II genes and is indepedent of protein synthesis.

Taken together, these results suggest that IAX is an important DNA binding protein for the expression of the MHC class II genes. Interestingly, IAX is the first MHC class II transcription factor known that shows a cell specific expression and is induced by interferon γ.

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Self-release and DM-mediated removal of CLIP and other HLA-DR associated self-peptides

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After biosynthesis MHC class II molecules associate with the monomorphic invariant chain (Ii) which is required for assembly and transport of MHC class II molecules. In endosomal compartments, stepwise removal of Ii takes place with the CLIP fragment still occupying the class II peptide binding groove. We could show that CLIP(81-105) can be subdivided into two functionally distinct regions: while the C-terminal segment (Ii90-105) is responsible for inhibition of peptide binding, the N-terminal segment (Ii81-89) mediates fast dissociation of CLIP which is further enhanced at endosomal pH. The nonameric CLIP fragment CLIP(81-89) even released CLIP(90-105) in trans as well as a subset of other self-peptides, probably by transient interaction with an effector site outside the groove. Similarly, the non-classical MHC class II molecule HLA-DM catalyzes removal of certain self-peptides including CLIP suggesting a peptide editing function. In this case, especially suboptimal anchors for the P1 pocket of the peptide binding groove confer high DM susceptibility.

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List of Invited Speakers

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Workshop on

MECHANISMS OF EXPRESSION AND FUNCTION OF MHC CLASS II MOLECULES

List of Invited Speakers

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MECHANISMS OF EXPRESSION AND FUNCTION OF MHC CLASS II MOLECULES

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